MECHANICAL PROPERTIES OF PINNA ADDUCTOR MUSCLE

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(Text-figs. 1-6)

INTRODUCTION

It is well known that the smooth adductors of lamellibranch molluscs can hold the shells closed against the tension exerted by the elastic hinge ligament for prolonged periods without visible signs of fatigue. Two opposing hypotheses have been put forward to explain this phenomenon. One postulates that tonic contraction is a tetanic phenomenon and that the economy of lamellibranch smooth muscles is due to their slow speed of relaxation (Ritchie, 1928); whilst the other proposes the existence of a 'catch mechanism' which enables tension to be maintained without expenditure of energy, so that no excitation is needed during tonic contraction (Jordan, 1938).

The mechanical properties of the smooth adductor of *Pinna* have recently been studied in detail by Reichel (1952, 1955) and Bandmann & Reichel (1954), who take the view that though there exists a 'plastic catch-tonus' (based on a mechanism different from that for phasic contraction) continuous excitation is needed to maintain that tonus.

In the experiments described by Bandmann & Reichel, treatment of the smooth posterior muscle of *Pinna* with acetylcholine (ACh) leads to the development of tonus, but it takes about 1 h for the tension to reach maximum. In similar experiments with *Mytilus* muscle we found that maximum tension was reached within 15 sec. This discrepancy, together with Bandmann & Reichel's observation that the *Pinna* muscle was always strongly contracted after isolation—whereas this is not true of *Mytilus* preparations—led us to investigate the *Pinna* adductor.

METHODS

The posterior adductor muscle of *P. fragilis* (Pennant, 1777, Edition 4, Vol. 4, p. 97 and pl. 69) was used. It consists of a small white portion facing the hinge and of a larger portion, darker in appearance and containing the longest fibres. Bandmann & Reichel (1954), who worked with *P. nobilis*, do not mention the presence of two macroscopically distinct portions in the posterior adductor. Strips of the muscle containing the longest fibres (dark portion) have been used in the present experiments and these correspond to the preparation described by Bandmann & Reichel.

Histologically, both portions are composed of unstriated fibres and the only distinction between them under the light microscope is that the fibres of the dark portion have a slightly smaller diameter than those which compose the white portion.

Specimens of *P. fragilis* were collected from the region of the Grande Vasière near Concarneau (Brittany). They were kept in running sea water in the Plymouth aquarium and survived satisfactorily for up to 6 months. The posterior adductor muscle was exposed by removing parts of the shells with bone forceps. The visceral ganglia were quickly extirpated. After soaking in aerated sea water for about an hour the muscle relaxed completely under a small load. The length of the muscle with closed shells was from 25 to 30 mm; and relaxed with shells gaping, from 35 to 45 mm. Strips about 4 mm broad and 2 mm thick were prepared from the dark part, tied at both ends and cut. Sea water was used as a bathing medium.

These muscle strips relaxed under a light load to the longest length reached by them in the animal with shells gaping (*reference length*). If the muscle strips were pre-shortened by stimulation below the reference length, allowed to relax, and then stretched, a tension developed which decayed completely within about I min. This phenomenon was observed in all smooth and striated muscles which we have investigated (Abbott & Lowy, unpublished) and shows that no resting tension is developed below reference length.

Muscle strips were mounted on a multielectrode stimulating assembly (Hill, 1949) and connected by a light silver chain to an isometric or isotonic lever. Stimulation was by square wave pulses from an electronic stimulator (Attree, 1950). The strength-duration curve for single twitches (threshold responses) was obtained in order to find a suitable pulse width for stimulation. The width generally used was 3 msec, with a voltage of 10 V for maximal stimulation. Contraction was recorded photoelectrically and displayed on a cathoderay tube.

Records at room temperature were taken of isometric twitches, isometric tetani and of after-loaded isotonic contractions. The stimulus frequency for tetanus was determined from fusion frequency experiments.

The mechanical properties of the resting muscle were investigated by stretches which were applied by lowering a Palmer stand. The resulting tension changes with time were recorded.

Responses to acetylcholine were studied. A final concentration in the bath of 10^{-5} or 10^{-6} g/ml. was adequate to produce maintained tonic contractions.

Some preliminary experiments have been done on the mechanical properties of the small white portion of *Pinna's* posterior adductor: it is much slower than the darker part of the muscle. The latter will from now on be referred to as the fast part of the adductor.

RESULTS

Excitability

Isolated strips of the fast part of *Pinna's* posterior adductor muscle respond to a single stimulus with a twitch. The strength-duration curve for the system is shown in Fig. 1. On the evidence available it is not possible to decide whether this represents excitation of nerve or muscle. Using either multiplepoint or one-end stimulation, a maximal isometric twitch can be obtained (Fig. 2). At the recording sensitivity used, the first sign of tension appears at 40 msec (latent period) and the peak twitch tension of 250 g/cm² is reached in 200 msec. Relaxation is very slow compared with the rising phase, tension falling to half-value in 2 sec.



Fig. 1. Strength-duration curve: threshold responses to single stimuli.

Repeated stimuli give responses which summate. There are no signs of facilitation (Fig. 3). The fusion frequency is 7/sec at room temperature and the tetanus tension is about 6 times that developed during a twitch. An isometric tetanus at 7/sec shows a smooth rise of tension which reaches maximum within 4 sec. When stimulation ends the muscle relaxes completely.

Isotonic Shortening

The speed of shortening under afterload conditions varies with load as shown in Fig. 4, the load for zero speed being represented by the isometric tension. The curve is a hyperbola and can be described by Hill's (1938) characteristic relation between force and velocity:

 $(P+a)(v+b) = b(P_0+a),$

where P is load on the muscle, P_0 is isometric tension, v is speed of shortening, and a and b are constants.

In the muscle illustrated maximum speed of shortening is 8.5 mm/sec or 0.3 length/sec; it is faster than the *Mytilus* adductor (0.1 length/sec). The



Fig. 2. Record of isometric twitch with multi-point stimulation. Muscle strip, weight 240 mg, length 37 mm, temperature 15° C. Marks of the trace recorded every 20 msec, shock at origin of trace. Time base continuous, tension followed for $1\frac{1}{2}$ sweeps.



Fig. 3. Repeated twitches, 2/sec. Muscle strip as in Fig. 2.

maximum tension developed by the *Pinna* muscle is only 1.5 kg/cm^2 compared with about 2 kg/cm² for many other muscles (Wilkie, 1954), but this is probably due to damage during preparation. The value for a/P_0 is 0.35—well within the range found for a variety of muscle types (Wilkie, 1954).

Length-Tension Relations

The relaxed muscle at reference length shows no resting tension. Tetanic stimulation produces active tension and the value of peak isometric tension decreases on either side of an optimum, at about reference length (Fig. 5).

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Fig. 4. Force-velocity curve for muscle strip. Weight 250 mg, length 30 mm, temperature 15° C.



Fig. 5. Isometric tension-length curve for muscle strip. Temperature 15° C.

Fig. 6. Decay of tension following stretch of resting muscle strip above reference length. Reference length 35 mm, muscle stretched from 35 to 40 mm. In order to measure active tension at lengths greater than reference length allowance had to be made for resting tension which appears when the muscle is stretched above that length and decays with time as shown in Fig. 6. It was found that at such lengths the resting tension never declines to zero. The procedure adopted was to stimulate the muscle and measure tetanic tension 5 min after it had been extended, by which time the resting tension had fallen to an almost steady value. This gives the right-hand limb of the curve in Fig. 5.

If a long interval was allowed between each stretch so that tension decayed considerably, the muscle could be stretched to about 40-60% above reference length before it showed signs of tearing. The tearing stress is of the order of 6 kg/cm^2 .

We find that in *Pinna* muscle, as in *Mytilus* muscle (Abbott & Lowy, 1953), the resting and active length-tension curve can be shifted along the length axis. If the muscle is stretched well beyond its reference length, say by 25%, the tension on it allowed to decay considerably, and the active isometric length-tension curve again determined, its maximum is found to be no longer at the reference length but at a new longer length. However, the value of the peak tension is still the same, i.e. the curve has evidently moved along the length axis. This shift is irreversible and probably due to a 'slip' within the muscle. A similar phenomenon has been observed in vertebrate skeletal muscle (Aubert, Roquet & Van der Elst, 1951).

Tonic Contraction

The *Pinna* muscle responds to application of 10^{-6} ACh by a tonic contraction with up to 75% of maximal isometric tension. The plateau is reached within about 5 sec from the first sign of tension rise. Tension is well maintained for long periods in the presence of ACh but drops gradually to zero after the drug is washed out.

DISCUSSION

Bandmann & Reichel's (1954) hypothesis of the contractile mechanism in lamellibranch smooth muscle may be summarized as follows. Contraction produces shortening and is accompanied by disorientation of fibre, fibrillar and molecular elements within the muscle. This disorientation is long-lasting and associated with an increase in dynamic stiffness due to internal frictional forces. When the muscle is now stretched, these frictional forces resist extension and a state is set up which Bandmann & Reichel define as 'plastic tonus'.

In the animal, excitation causes the adductor to contract against the elastic tension exerted by the hinge ligament. It is assumed that when excitation stops, the frictional forces due to disorientation produced by contraction can 'take over' and resist the pull of the ligament by tension due to 'plastic tonus' (Sperrtonus or catch mechanism). This tension is believed to be the same as that which results from stretch of a resting, shortened, disorientated muscle; it decays extremely slowly and therefore in the animal very little re-excitation is needed to maintain it.

Evidence for the above hypothesis comes from Bandmann & Reichel's experiments on the mechanical properties of Pinna muscle. Length-tension relations of the isolated resting muscle were studied. Stretch produced a tension which decays very slowly. The object of this experiment was to demonstrate the existence of 'plastic tonus'. Next, the decrease in tension as the muscle was allowed to shorten from the stretched condition was investigated. A welldefined length-tension curve could be obtained on which zero tension was reached at the original starting length. But the experiment also showed that 'plastic tonus' can give only a limited amount of tension: if too great a stretch is applied, the muscle 'slips', i.e. if the length-tension curve is again determined, zero tension is reached at a new, longer length. This process, called 'plastic lengthening', is believed to be associated with re-orientation of muscle structures. It is of critical importance to Reichel's hypothesis, for 'plastic tonus' can only disappear by such re-orientation. Thus, in the animal, when the shells gape the muscle is supposed to be plastically lengthened; contraction to result in closure; and 'plastic tonus' to keep the shells closed. That this sequence of events is possible was demonstrated by Bandmann & Reichel in experiments where a plastically lengthened muscle was treated with acetylcholine. Tension was developed and when such an acetylcholineactivated muscle was allowed to shorten in steps and the recovery of tension observed, the length at which no tension was re-developed proved to be the original starting length, i.e. the length before 'slip' occurred.

Taking Bandmann & Reichel's experimental findings as they stand, their hypothesis is both attractive and consistent. But from our experience with other molluscan muscles we were concerned about three observations reported by Bandmann & Reichel. First, that after isolation the *Pinna* muscle was stated to be in a very contracted state, and, even after stretching with a heavy load for a matter of hours, could never be brought back to its body length. Secondly, that at all lengths stretch of the resting muscle resulted in a very long-lasting tension. Thirdly, that the time taken for development of maximum isometric tension following treatment with acetylcholine was about 1 h.

Our experimental results described above indicate that the *Pinna* muscle does in fact behave very much like other molluscan muscles. After suitable dissection it relaxes to maximum body length (reference length); any tension produced by stretch of the resting muscle at lengths below reference length decays completely within a minute; lastly, the time taken to reach maximum isometric tension, whether induced tetanically or by acetylcholine, is only of the order of seconds.

It would thus seem that the muscles used by Bandmann & Reichel were not in good condition. This is substantiated by our observation that if the ganglia are not extirpated rapidly, molluscan smooth muscles often go into a state of 'contracture' and then give much the same responses as those described by Bandmann & Reichel. We have also found that when muscle strips are prepared complete with shell attachments, similar 'contractures' may develop—presumably due to violent excitation whilst cutting the shell close to the muscle strip.

Similar considerations possibly apply to the experiments of Brecht, Utz & Lutz (1955) with *Anodonta* adductors in which the muscles were slow to contract and failed to relax completely after tetanic stimulation. These workers investigated oxygen consumption polarographically and found that the increased rate of metabolism associated with contraction returns to its resting value whilst tension still persists. Brecht and his collaborators conclude from this that a 'freezing' of the contractile elements (catch mechanism) could be responsible for maintenance of tension without increased oxygen consumption. This is contrary to our own findings with *Mytilus* muscle where increased energy expenditure (heat production) accompanies tonic contraction (Abbott & Lowy, 1955).

In our view, the economy of lamellibranch smooth muscle is due to its slow rate of relaxation. The present work on Pinna serves to support this hypothesis which has been mainly derived from experiments on Mytilus muscle (Lowy, 1953; Abbott & Lowy, 1953, 1955; Hoyle & Lowy, 1956). The fast Pinna adductor proved to be much quicker than Mytilus muscle; its intrinsic speed is actually comparable to that of some vertebrate striated muscles. On the other hand, as is the case in most other molluscan smooth muscles, the Pinna adductor relaxes relatively slowly. Peak twitch tension is reached by 200 msec, but relaxation is complete only after 12 sec. These findings can be considered in terms of the concept of active state (Hill, 1949). The level of active state in a muscle is equated to the tension which the contractile component can exert if it is neither shortening or lengthening. Full activity is developed very soon after stimulation (as shown by quick-stretch experiments). This active state decays with time and at the peak of an isometric twitch, when the contractile and series elastic elements are in equilibrium, the tension exerted represents the level of active state remaining. Since in *Pinna* muscle the tetanus-twitch ratio is about 6, only 1/6 of the full active state remains 200 msec. after the stimulus. Tension, however, decays to zero only after 12 sec. Thus the contractile component returns to a state of complete rest a very long time after the active state has disappeared.

From the above considerations we conclude that tension in the intact *Pinna* adductor cannot be identified with tension due to stretch of isolated resting muscle, i.e. 'plastic tonus' is not a physiological phenomenon. Bandmann & Reichel (1954) and Reichel (1955) agree with the view (Lowy, 1953) that re-excitation is needed to maintain tonic contraction but their concept of the mechanism involved differs basically from our own. Whereas their 'plastic

tonus' reflects an internal state of disorientation in resting muscle, we explain the economy of tonic contraction in lamellibranch smooth muscle by the slowness with which the contractile component returns from the active to the resting state.

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SUMMARY

The mechanical properties of the fast portion of *Pinna's* posterior adductor muscle at rest and during activity have been studied.

The muscle responds to a single shock with a twitch and to repeated shocks with a tetanus. Maximum speed of shortening is about 0.3 length/sec. and peak isometric twitch tension is reached at 200 msec. Relaxation is slow, tension dropping to half in 2 sec and to zero in 12 sec.

Acetylcholine induces a tonic contraction, peak tension being reached in 7 sec. Tonus is only maintained as long as the drug is present.

Contraction in lamellibranch smooth muscle is discussed in the light of Reichel & Bandmann's (1954) hypothesis. On this hypothesis, tonus in the intact muscle is supposed to be maintained by the same mechanism which produces long-lasting tension when resting isolated muscle is stretched ('plastic tonus'). The present experiments show that tension produced by stretch of isolated resting muscle within the limits of body lengths is longlasting only if the muscle is in poor condition. From this evidence on time relations of muscles in good and poor condition, it is concluded that 'plastic tonus' is not a physiological phenomenon.

The tetanus hypothesis of tonic contraction in lamellibranch smooth muscles is supported, and the economy of these muscles is explained in terms of their slow relaxation rate.

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